PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BOR00006/WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2004/000282	International filing date *(day/month/year)* 23.04.2004	Priority date *(day/month/year)* 23.04.2003
International Patent Classification (IPC) or national classification and IPC C07K19/00, C12N9/64		
Applicant BOREAN PHARMA A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.11.2004	06.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Telephone No. +49 89 2399-

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000282

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-65 as originally filed

Sequence listings part of the description, Pages

1-26 as originally filed

Claims, Numbers

1-39 as originally filed

Drawings, Sheets

1/21-21/21 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-39
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-39
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-39
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item IV.

The separate inventions/groups of inventions are:

Group I: Claims 1-32(all complete),36-39(all partial)
A method for the preparation of a fusion protein comprising (I) from N- to C-terminus (a) a fusion partner, (b) a Granzyme B protease cleavage site, c) a polypeptide of interest, wherein the cleavage site is adjacent to the polypeptide of interest, and
(ii) contacting said fusion protein with Granzyme B protease to cleave it and yield a protein of interest.
A fusion protein comprising (I) from N- to C-terminus (a) a fusion partner, (b) a Granzyme B protease cleavage site, c) a polypeptide of interest, wherein the cleavage site is adjacent to the polypeptide of interest.

Group II: Claims 33-35(all complete), 36-39(all partial)
A human Granzyme B protease variant wherein the Cysteine residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The present application discloses in claim 1 a method for producing a protein of interest by constructing a fusion protein comprising a Granzyme B protease cleavage site between the fusion partner and the protein of interest from N- to its C-terminus and cleaving said construct with Granzyme B. Moreover, a fusion protein is claimed consisting of a Granzyme B protease cleavage site between the fusion partner and the protein of interest from N- to its C-terminus (claim 18). In addition, a Granzyme B protease variant is claimed containing a particular point mutation (claim 33). Fusion proteins containing a protease cleavage site and Granzyme B protease in general and its cleavage site is known from the prior art (see page 2, fourth para. to page 3, second para. and page 7, third para. to page 9, first paragraph of the present application).
In the light of the prior art, the problems underlying the present application can be seen as the provision of an alternative method for producing proteins of interest from a fusion protein construct and the provision of increased amounts of Granzyme B.

The solution can be summarised as the provision of the method of claim 1 and the product of claim 18 and on the other side the provision of the particular Granzyme B point mutation. The only linking concept between the method claimed, the fusion protein and the Granzyme B point mutation is the Granzyme B protease itself. However, Granzyme B protease and its cleavage site were already known from the prior art (see Harris et al, JBC, vol. 273, pg.: 27364-27373, abstract; Sun et al., JBC, vol. 276, 2001, pg.: 15177-15184, abstract). Moreover, no other technical feature can be distinguished which in the light of the prior art could be regarded as a special common technical feature linking the two different groups of inventions mentioned above. Thus, the ISA is of the opinion that there is no single inventive concept underlying the plurality of different inventions in the sense of Rule 13.2 PCT. Consequently, there is a lack of unity and the different inventions not belonging to a common inventive concept are formulated as different groups of inventions pursuant to Art. 17(3)(a) PCT. The following opinion will refer to both inventions comprising the subject-matter of claims 1-39 since the search fees have been paid accordingly.

Re Item V.

The following documents are referred to in this communication:

D1 : HARRIS J L ET AL: JBC, 16 OCT 1998, vol. 273, no. 42,, pages 27364-2737
D2 : SUN JIURU ET AL: JBC, vol. 276, no. 18, 4 May 2001, pages 15177-15184
D3 : SUN JIURU ET AL: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 261, no. 2, 2 August 1999, pages 251-255
D4: SUN JIURU ET AL: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 261, no. 2, 2 August 1999, pages 251-255
D5: ROTONDA J ET AL: MOLECULAR CELL, vol. 8, no. 4, April 2001, pages 357-368

1. Claim 1 refers to a method for the preparation of a fusion protein comprising (I) from N- to C-terminus (a) a fusion partner, (b) a Granzyme B protease cleavage site, © a polypeptide of interest, wherein the cleavage site is adjacent to the polypeptide of interest, and

(ii) contacting said fusion protein with Granzyme B protease to cleave it and yield a protein of interest.
Claim 18 refers to a fusion protein comprising (I) from N- to C-terminus (a) a fusion partner, (b) a Granzyme B protease cleavage site, © a polypeptide of interest, wherein the cleavage site is adjacent to the polypeptide of interest.
Claim 33 refers to a human Granzyme B protease variant wherein the Cysteine residue no. 228 is mutated to Phenylalanine.

None of the available prior art documents disclose either the method of claim 1, the fusion protein of claim 18 nor the Granzyme B variant of claim 33. Thus, the subject-matter of claims 1, 18 and 33 is considered to be novel (Article 33(2) PCT). The same applies to the subject-matter of claims 2 to 17, 19 to 32, 34 to 39 dependent thereon.

2. Moreover, the method of claim 1, the fusion protein of claim 18 and Granzyme B variant of claim 33 appear to be inventive for the following reasons:

D3 is considered to be the closest prior art. The document discloses a method for the production of human Granzyme B by using a fusion protein comprising maltose binding protein and granzyme B separated by an enterokinase cleavage site. The cleavage was said to be precise and generated an active recombinant granzyme B (see abstract, page 253, col. 1, second para. to col. 2, first para.). The subject-matter of present claim 1 and 18 is distinguished therefrom by using a granzyme B protease cleavage site instead. This difference results in an alternative method and fusion protein for the production of proteins of interest.
The problem to be solved by the present application was thus to provide an alternative method and fusion protein construct for the production of proteins of interest.
The use of the granzyme B cleavage site has the advantage that not only no spurious or extraneous amino acids are left to the cleaved protein of interest but also that the cleavage site motif is more flexible allowing more flexibility with respect to the production of recombinant proteins in general. The cleavage site and the substrate specificity of granzyme B was known from the prior art (see D1 and D2, abstract). However, the use of granzyme B for the production of recombinant proteins was

neither known nor was its use indicated or pointed at in any of the documents cited neither alone nor in any combination. Thus, presence of an inventive step could be acknowledged (Article 33(3) PCT). The same applies to the subject-matter of claims 2 to 17, 19 to 32 and 36 to 39 dependent thereon.

D4 is considered to be the closest prior art for the subject-matter of claim 33. This document reveals a recombinant expression of the wild type Granzyme B in yeast. The subject-matter of claim 33 is distinguished therefrom by referring to a particular point mutation at position 228 of the enzyme. This mutation results in a higher recombinant expression rate.

The objective problem to be solved by the present application was thus to increase the expression rate of recombinant Granzyme B.

The problem was solved by the particular point mutation of claim 33. This increased expression rate could not be predicted from any of the available prior art documents, neither alone nor in any combination. Thus, presence of an inventive step can be acknowledged for the subject-matter of claim 33 (Art. 33(3) PCT). The same applies to the subject-matter of claims 34 to 39 dependent thereon.

3. The following matter requires attention:

3.1 Claims 1 and 18 refer only broadly to the functional term "granzyme b protease recognition site". This renders the claims vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT. The applicant should incorporate the subject-matter of claim 2 into said claims.

3.2 The scope of protection of claim 35 is vague and unclear (Art. 6 PCT).